PATENT
Our Docket: P41 9498

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE
BEFORE THE BOARD OF APPEALS AND INTERFERENCES

In re application of: Wahl and O'Gorman	Group Art Unit: 1814
Serial No.: 08/147,912	Examiner: C. Low
Filed: November 3, 1993	
For: FLP-MEDIATED GENE MODIFICATION IN MAMMALIAN CELLS, AND COMPOSITIONS AND CELLS USEFUL THEREFOR	

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APPELLANT'S REPLY BRIEF UNDER 37 CFR 1.193(b)

Sir:

I. INTRODUCTION

This is a reply to the Examiner's Answer, based on Appellants' appeal from a decision of the Examiner dated July 14, 1994, finally rejecting pending claims 25, 26, 28, 42-46 and 48 in the above-identified patent application. Notice of Appeal was timely filed January 17, 1995. The Appeal Brief was timely filed on July 17, 1995. The Examiner's Answer was mailed on December 12, 1995. This Reply Brief is being submitted in triplicate (an original and two copies) as required under 37 C.F.R. 1.192(a).

II. REAL PARTY IN INTEREST

This remains the same as previously set forth in Appellants' Brief.

III. RELATED APPEALS AND INTERFERENCES

This remains the same as previously set forth in Appellants' Brief.

IV. STATUS OF THE CLAIMS ON APPEAL

The present application, USSN 08/147,912, filed November 3, 1993, is a file-wrapper continuation of USSN 07/666,252, filed March 8, 1991.

The original application ('252) contained claims 1-59. Claims 1-24, 29-41 and 56-59 were withdrawn from consideration as a result of Appellants' election in response to the Requirement for Restriction mailed July 17, 1992.

By an amendment and request for reconsideration filed March 12, 1993 in connection with '252, claims 25-28, 42-44, 47-51 and 54-55 were amended. On June 3, 1993, the Examiner issued an Office Action finally rejecting claims 25-28 and 42-55.

On October 18, 1993, Appellants filed an amendment after final and request for reconsideration wherein claims 25, 26, 28, 42-46 and 48 were amended and claims 27, 47 and 49-55 were canceled.

An Advisory Action was issued October 26, 1993 in connection with '252, stating that Appellants' request for reconsideration was considered but was deemed not to overcome the grounds for rejection. The amendments proposed on October 18, 1993 were not entered.

On November 3, 1993, Appellants filed the present application as a file-wrapper-continuation of original application '252. In the concurrently filed preliminary amendment, non-elected claims 1-24, 29-41 and 56-59 were canceled, and the amendments proposed on October 18, 1993 in connection with '252 were entered in the present application. Thus, claims 25, 26, 28, 42-46 and 48 are pending in the present application.

By an amendment and request for reconsideration filed May 9, 1994, claims 25, 26, 28, 42-44 and 48 were further amended. On July 14, 1994, the Examiner issued an Office Action finally rejecting claims 25, 26, 28, 42-46 and 48.

Appellants filed a Notice of Appeal on January 17, 1995, from the final rejection of claims 25, 26, 28, 42-46 and 48. On July 17, 1995, Appellants filed an Amendment After Final and request for reconsideration wherein proposed amendments to claims 25, 26, 42, 44 and 48 were presented.

An Advisory Action was issued on August 24, 1995, stating that upon the filing of an Appeal, the proposed amendments to claims 25, 26, 42, 44 and 48, proposed in the July 17, 1995, Amendment After Final, would not be entered, and that Appellants' request for reconsideration was considered but was deemed not to overcome the grounds for rejection of the claims.

Accordingly, pending claims 25, 26, 28, 42-46 and 48, as they stood prior to Appellants' July 17, 1995, Amendment After Final, define the subject matter of this Appeal. A copy of claims 25, 26, 28, 42-46 and 48 is presented in Appendix A.

V. STATUS OF AMENDMENTS

An Amendment After Final under 37 C.F.R. § 1.116 was submitted on July 17, 1995. Amendments to claims 25, 26, 42, 44 and 48 were proposed in response to the issues raised in the Official Action dated July 14, 1994. In the Advisory Action issued on August 24, 1995, Appellants were advised that the

request for reconsideration was deemed not to overcome the grounds for rejection of the claims and that upon the filing of an Appeal, the amendments to claims 25, 26, 42, 44 and 48, proposed on July 17, 1995, would not be entered.

VI. SUMMARY OF INVENTION

In accordance with the present invention, there are provided methods for the site-specific integration of DNA into the genome of a cell. The specific site of integration is referred to as an "FLP recombination target site" (i.e., an "FRT"). Because the FRT is a relatively short sequence of nucleotides, it can be integrated into the DNA of a host without unduly disrupting the host's normal processes. Once the FRT is integrated at a confirmed site of interest, employing standard techniques, the targeted integration of a desired construct provided by the FLP/FRT recombination system used in accordance with the claimed methods alleviates the randomness commonly associated with the transfection of DNA.

The novel site-specific recombination system of the present invention provides the artisan with the ability to target the integration of transfected DNA to specific chromosomal sites in mammalian host cells at frequencies substantially exceeding those of both random and other site-specific integration systems.

Additionally, invention recombination system allows for immediate confirmation and analysis of the recombination event. The recombination system described herein is distinctive in its precision and predictability, providing methods which enable the artisan to routinely create or disrupt functional translational reading frames at intended sites of integration.

VII. ISSUES

Issues 1-6 remain the same as previously set forth in Appellants' Brief.

7. Is the Examiner's new point of argument, advanced for the first time in the Examiner's Answer, asserting that the second DNA may recombine randomly in the genome at sites other than the FLP recombination target site, supported by any credible evidence?

VIII. GROUPING OF CLAIMS

This remains the same as previously set forth in Appellants' Brief.

IX. ARGUMENT

1. The Examiner has raised a new point of argument.

In the Examiner's Answer it has been asserted that "the second DNA may or may not also recombine randomly at other sites in the genome of the cell." See Paper No. 36, p. 3, lines 8-9; also see p. 3, lines 23-24, and p. 5, lines 27-29. This is a new point of argument not previously raised in any of the Office Actions in this case. See Paper Nos. 24, 26, and 33. Since this point of argument was not previously raised, Appellants are entitled to respond pursuant to 37 CFR 1.193(b).

2. The Examiner's new point of argument is not supported by any credible evidence.

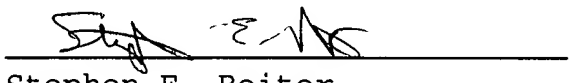
Appellants respectfully disagree with the Examiner's assertion that the second DNA may recombine randomly at other sites in the genome of the cell (i.e., at sites other than the site of the FRT sequence inserted into the genome of the cell by step (i) of the claimed process). It is respectfully submitted to be highly unlikely for such an event to occur, especially in view of the high selectivity demonstrated by Appellants for the FLP recombinase system. Furthermore, no evidence of record supports the Examiner's assertion. In contrast, Appellants have

shown that the recombination catalyzed by FLP recombinase is highly specific for DNA sequences that contain the FRT sequences. See, for example, Appellants' specification at p. 12, lines 5-17.

X. CONCLUSION

Accordingly, in view of the above remarks, as well as the arguments set forth in Appellants' Brief on Appeal, Appellants respectfully submit that claims 25, 26, 28, 42-46 and 48 are in condition for allowance. Therefore, Appellants respectfully request that the decision of the Examiner, finally rejecting claims 25, 26, 28, 42-26 and 48, be reversed.

Respectfully submitted,



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APPENDIX A

The text of pending claims on appeal are:

25. (Amended) A method for precisely targeting integration of a nucleic acid into the genome of a mammalian host cell, said method comprising:

(i) stably integrating a first nucleic acid comprising a FLP recombination target site (FRT) into the genome of said mammalian host cell,

(ii) introducing into said mammalian host cell of step (i) a second nucleic acid comprising at least one FRT along with an FLP recombinase, wherein said FLP recombinase catalyzes recombination between the integrated FRT and the FRT of said second nucleic acid, thereby precisely targeting integration of said second nucleic acid into the genome of said mammalian host cell of step (i).

26. (Amended) A method for excising a second nucleic acid that has been integrated into the genome of a mammalian host cell according to the method of Claim 25, comprising contacting the genomic DNA of said mammalian host cell with an FLP recombinase, wherein said FLP recombinase catalyzes recombination of the FRT of said first nucleic acid and the FRT of said second nucleic acid, thereby excising the integrated second nucleic acid from the genome of said mammalian host cell.

28. (Amended) A method according to Claim 25, further comprising introducing into the mammalian host cell of step (ii) a third nucleic acid comprising at least one FRT, along with an FLP recombinase, wherein said FLP recombinase catalyzes recombination between an integrated FRT with the FRT of said third nucleic acid, thereby precisely targeting integration of said third nucleic acid into the genome of said mammalian host cell.

42. (Amended) A method for the site-specific integration of a nucleic acid into the genome of a mammalian cell wherein at least one FRT is stably integrated in the genome of said mammalian cell, said method comprising:

introducing into said mammalian cell a first nucleic acid comprising at least one FRT and at least a first partial coding sequence of a first gene of interest, along with an FLP recombinase, wherein the FLP recombinase catalyzes recombination between the integrated FRT and the FRT of said first nucleic acid, thereby specifically integrating said first nucleic acid at the site of FRT recombination in said genome of the mammalian cell.

43. (Amended) A method according to Claim 42, wherein said FRT(s) integrated in the genome of said mammalian cell is/are positioned within the protein coding sequence of said gene of interest.

44. (Amended) A method according to Claim 42, further comprising contacting said mammalian cell with a second nucleic acid comprising at least one FRT and at least a second partial coding sequence of the first gene of interest or a partial coding sequence of a second gene of interest, along with an FLP recombinase, wherein the FLP recombinase catalyzes recombination between said integrated FRT and the FRT of said second nucleic acid, wherein said second nucleic acid specifically integrates at the site of FRT recombination in reading frame with said first nucleic acid, wherein the combination of said first and said second nucleic acids provides a functional gene.

45. A method according to Claim 42 wherein said FLP recombinase is provided by a FLP expression vector.

46. A method according to Claim 45 wherein the expression of FLP recombinase by said FLP expression vector is subject to regulatory control.

48. (Amended) A method according to Claim 42, further comprising contacting said mammalian cell with a second nucleic acid comprising at least one FRT, along with an FLP recombinase, wherein the FLP recombinase catalyzes recombination between said integrated FRT and the FRT of said second nucleic acid, wherein said second nucleic acid specifically integrates at the site of FRT recombination and combines with said first nucleic acid, wherein the combination of said first nucleic acid and said second nucleic acid prevents expression of the first gene of interest.